

## Polymorphic Markers in the *SRD5A2* Gene and Prostate Cancer Risk: A Population-based Case-control Study<sup>1</sup>

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### Abstract

It has been suggested that the activity of the steroid 5 $\alpha$ -reductase type II enzyme (encoded by the *SRD5A2* gene) may be associated with prostate cancer risk and that population differences in this enzyme's activity may account for part of the substantial racial/ethnic disparity in prostate cancer risk. To provide etiological clues, we evaluated the relationships of four polymorphic markers in the *SRD5A2* gene, specifically, *A49T* (a substitution of threonine for alanine at codon 49), *V89L* (a substitution of leucine for valine at codon 89), *R227Q* (a substitution of glutamine for arginine at codon 227), and a (*TA*)<sub>n</sub> dinucleotide repeat, with prostate cancer risk in a population-based case-control study in China, a population with the lowest reported prostate cancer incidence rate in the world. Genotypes of these four markers were determined from genomic DNA of 191 incident cases of prostate cancer and 304 healthy controls using PCR-based assays, and serum androgen levels were measured in relation to these genotypes. All study subjects had the wild-type *AA* genotype of the *A49T* marker, and 99% had the *RR* genotype of the *R227Q* marker. For the *V89L* marker, prevalences of the *LL*, *VV*, and *VL* genotypes among controls were 35%, 21%, and 45%, respectively. Compared with men with the *VV* genotype, those with the *LL* genotype had a statistically nonsignificant 12% reduced risk (odds ratio = 0.88, 95%

confidence interval, 0.53–1.47). In addition, men with the *LL* genotype had significantly higher serum levels of testosterone and significantly lower serum levels of 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol glucuronide than men with other genotypes. Men heterozygous for the (*TA*)<sub>o</sub> allele of the (*TA*)<sub>n</sub> marker had a modest, statistically nonsignificant risk reduction (odds ratio = 0.67; 95% confidence interval, 0.39–1.12) compared with men homozygous for the (*TA*)<sub>o</sub> allele, along with significantly higher serum dihydrotestosterone levels. The observed *V89L* genotype prevalences and the association between *V89L* genotypes and serum androgen levels support the hypothesis that genotypes associated with lower levels of 5 $\alpha$ -reductase activity are more common in low-risk populations. Although we found no statistically significant associations of these *SRD5A2* polymorphisms with prostate cancer risk, a small effect of these markers cannot be ruled out because of the rarity of certain marker genotypes. Larger studies are needed to further clarify the role of these markers and to elucidate whether genetic diversity of the *SRD5A2* gene, alone or in combination with other susceptibility genes, can help explain the large racial/ethnic differences in prostate cancer risk.

### Introduction

Prostate cancer is the most commonly diagnosed non-skin cancer and is the second leading cause of cancer death in men in the United States (1). Despite the fact that the prevalence of latent prostate tumors appears to be similar in most populations (2), there is a marked difference in the incidence of clinical prostate cancer, with African Americans having incidence rates that are 30–50 times higher than those of Asians living in Asia (3). The reasons for the racial/ethnic differences in incidence are unclear.

There is compelling evidence supporting a role of androgens in prostate carcinogenesis (4). Androgens are required for prostate growth, and administration of T<sup>3</sup> in laboratory rats can induce prostate tumors (5, 6). Within the prostate gland, the type II steroid 5 $\alpha$ -reductase enzyme irreversibly metabolizes T to DHT, which in turn binds to the androgen receptor, thus activating transcription of androgen receptor-responsive elements in target genes and inducing cellular proliferation (7). The type II steroid 5 $\alpha$ -reductase enzyme is encoded by the *SRD5A2* gene located on chromosome 2 (2p23; Ref. 8). The *SRD5A2* gene spans over 40 kb of genomic DNA, with 5 exons and 4 introns (9, 10). Although the enzyme has never been purified, over 22 mutations, including 10 single amino acid

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<sup>3</sup> The abbreviations used are: T, testosterone; SSCP, single-strand conformational polymorphism; DHT, dihydrotestosterone; 3 $\alpha$ -diol G, 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol glucuronide; OR, odds ratio; CI, confidence interval; SHBG, sex hormone-binding globulin; UTR, untranslated region.

missense substitutions, have been reported for the *SRD5A2* gene (11).

It has been suggested that differences in steroid 5 $\alpha$ -reductase type II enzyme activity may help explain part of the marked racial disparity in the incidence of clinical prostate cancer across populations (12). Indirect data supporting this hypothesis include the observations that, compared with Western men, Asian men have lower circulating levels of T and much less body hair [a phenotype that is probably related to the activity of the type I enzyme (13, 14)] and that racial variation in the allele frequencies of several *SRD5A2* markers associated with prostate cancer risk, including *V89L* (a valine to leucine substitution at codon 89) and  $(TA)_n$  (a TA dinucleotide repeat in the 3'-UTR of the gene), parallels the patterns of prostate cancer risk in high- and low-risk populations (15–17). For example, data from one genetic survey show that Asian men have a higher prevalence of the *LL* genotype of the *V89L* marker, which among Asians has been associated with lower serum levels of 3 $\alpha$ -diol G, an indirect measure of intraprostatic 5 $\alpha$ -reductase activity (15).

Two studies reported a positive association with the *A49T* marker (an alanine to threonine substitution at codon 49) in Latinos and African Americans (16, 18). However, other studies in Western and Japanese men failed to show a significant association of the *V89L* polymorphism with prostate cancer risk (19–21), and the  $(TA)_n$  marker was not significantly associated with risk among Western men (20, 22). To provide insights into the reasons for the racial/ethnic disparity in prostate cancer risk, in this report we evaluate the roles of the *V89L*, *A49T*,  $(TA)_n$  repeat, and *R227Q* (the arginine to glutamine substitution at codon 227) markers in prostate cancer in a multidisciplinary population-based case-control study in China. Serum androgen concentrations were also measured in relation to genotypes of the four polymorphisms. The first three markers were chosen because they have been implicated in prostate cancer etiology in previous studies (12). We investigated the *R227Q* polymorphism because the *RQ* variant has significantly reduced 5 $\alpha$ -reductase activity and to date has been observed only among Asians (11).

## Materials and Methods

**Study Subjects.** Details of the study have been described previously (23–25). Briefly, cases of primary prostate cancer (International Classification of Diseases 9 code 185) newly diagnosed between 1993 and 1995 were identified through a rapid reporting system established between the Shanghai Cancer Institute and 28 collaborating hospitals in urban Shanghai. Cases were permanent residents in 10 urban districts of Shanghai (henceforth referred to collectively as Shanghai) who had no history of any other cancer. Because prostate cancer screening is not widespread in China, most cases are men with clinically significant cancers who present with symptoms. Case ascertainment is estimated to be essentially complete because reporting to the Shanghai Cancer Registry by all Shanghai hospitals is mandatory.

Based on the personal registry cards of all adults over age 18 years residing in urban Shanghai (maintained at the Shanghai Resident Registry), male population controls were selected randomly from the 6.5 million permanent residents of Shanghai and frequency-matched to the expected age distribution (by 5-year category) of prostate cancer cases.

Information on potential risk factors was elicited through an in-person interview by trained interviewers using a structured questionnaire. The interview included information on

demographic characteristics, dietary and smoking history, consumption of alcohol and other beverages, medical history, family history of cancer, physical activity, body size, and sexual behavior. Of the 268 eligible cases (95% of the cases diagnosed in Shanghai during the study period), 243 (91%) were interviewed. After a consensus review by both the Chinese and American pathologists, four cases were classified as having benign prostatic hyperplasia and excluded from the study. Of the 495 eligible controls, 472 (95%) were interviewed. This study was approved by the Office of Human Subjects Research, NIH (Bethesda, MD) and the Institutional Review Board of the Shanghai Cancer Institute (Shanghai, China).

**Blood Collection and DNA Extraction.** Two hundred cases (82% of those interviewed) and 330 controls (70%) provided 20 ml of fasting blood for the study. The blood samples were processed at a central laboratory in Shanghai. The buffy coat fractions were first stored at  $-70^{\circ}\text{C}$ , and then shipped in dry ice to the American Type Culture Collection (Manassas, VA) for DNA extraction. Standard quality control procedures showed no evidence of contamination, and DNA purity and length were satisfactory. After DNA extraction, 191 cases and 305 controls had sufficient DNA for genotyping.

**Genotyping.** To minimize bias due to day-to-day laboratory variation, DNA samples were arranged in case-control pairs and triplets such that the same proportion of total cases and controls was assayed in each batch. Laboratory personnel were masked to case-control status. Capillary electrophoresis and allele-specific PCR analyses were used to determine the  $(TA)_n$  and *V89L* genotypes, whereas SSCP analyses were used to determine the *A49T* and *R227Q* markers (11, 15–17, 26).

***A49T*.** Genotypes at the *A49T* polymorphism were determined as described previously (16). Briefly, we amplified radiolabeled exon 1 of the *SRD5A2* gene [using the primers oNM16 (GCAGCGCCACCGGCG) and oNM32 (GTGGAAGTAATGTAGGCAGAA)] and then subjected it to SSCP analysis to determine genotypes at the *A49T* locus.

***V89L*.** The *V89L* polymorphism was analyzed using allele-specific PCR and the nonspecific forward primer (5'-AGGC-AGCGCCACTTTGG-3'), the nonspecific reverse primer (5'-TCGGTGCGCGCTCCACG-3'), and either the valine-specific (5'-CTACCTGTGGAAGTAATGTAC-3') or the leucine-specific (5'-CTACCTGTGGAAGTAATGTAG-3') primer. Amplifications were performed in a PE Applied Biosystems GeneAmp PCR System 9700 as follows: initial denaturation at  $95^{\circ}\text{C}$  for 2 min; 15 cycles of  $94^{\circ}\text{C}$  for 1 min,  $63^{\circ}\text{C}$  for 1 min, and  $72^{\circ}\text{C}$  for 2 min; 25 cycles of  $94^{\circ}\text{C}$  for 1 min,  $59^{\circ}\text{C}$  for 1 min, and  $72^{\circ}\text{C}$  for 2 min; and a final extension at  $72^{\circ}\text{C}$  for 5 min. PCR products were resolved in a 3% Nusieve agarose gel, stained with ethidium bromide, visualized under UV light, and photographed. Allele calling was performed independently by two researchers.

***R227Q*.** The *R227Q* polymorphism was assayed as described previously (11), using the primers oNM22 (h5a2-22; GCAATGATTGACCTTCCGATT) and oNM23 (GTTTGAGAGAAAGAAAGCTAC) in PCR reactions followed by SSCP analysis.

**TA Repeats.** The  $(TA)_n$  marker was assayed using PCR reactions containing the reverse primer (5'-GGCAGAACGCCAGGAGAC-3'), as well as both unlabeled and TET-labeled forward primer (5'-GAAAACTGTCAAGCTGCTG-3'). Cycling conditions were as follows:  $95^{\circ}\text{C}$  for 2 min; 35 cycles of  $94^{\circ}\text{C}$  for 1 min,  $55^{\circ}\text{C}$  for 1 min, and  $72^{\circ}\text{C}$  for 2 min; and  $72^{\circ}\text{C}$  for 5 min. Amplification was performed in a PE Applied Biosys-

tems GeneAmp PCR System 9700, and the number of (TA) repeats was determined using a Perkin Elmer ABI Prism 310 genetic analyzer and clones with known numbers of repeats as standards.

**Quality Control.** Twenty-four split samples from a single individual were spaced at intervals among the study samples to assess the reproducibility of genotyping. Of the 24 split samples genotyped for the *V89L*, *R227Q*, and (TA)<sub>n</sub> polymorphisms, all 24 were typed as *VL*, *RR*, and (TA)<sub>o</sub>, respectively. Of the 18 split samples assayed for *A49T*, 16 were typed as *AA*, whereas the other 2 had insufficient DNA.

**Serum Hormone Analysis.** Serum concentrations of sex hormones, including T, DHT, estradiol, and 3 $\alpha$ -diol G, and their binding protein, SHBG, were measured by RIA. Before RIA, T and DHT were extracted with hexane:ethyl acetate (3:2) and purified by Celite column partition chromatography as described previously (27, 28). Levels of 3 $\alpha$ -diol G and SHBG were measured directly in serum using highly specific antisera (29, 30). The intra-assay and interassay coefficients of variation for all analytes ranged from 4–8% and 10–13%, respectively.

**Statistical Analysis.** We compared frequencies of each of the marker genotypes by case control status. Unconditional logistic regression analyses were used to compute ORs and 95% CIs estimating the association of each of the *SRD5A2* markers with prostate cancer risk after adjusting for other potential risk factors, including education, smoking, alcohol consumption, body mass index, waist:hip ratio, and physical activity (31). We used overall F-tests from linear regression models to test for differences in mean age-adjusted serum sex hormone levels among controls across genotypes of each *SRD5A2* marker. All presented *P*s are two-sided.

## Results

Age at diagnosis ranged from 50–94 years (median age, 73 years) for cancer cases. About two-thirds of the cases were diagnosed as having advanced cancer (regional/remote stages), and most tumors were moderately or poorly differentiated. Most cases were symptomatic at diagnosis, with 77% of the cases having serum prostate-specific antigen levels greater than 10 ng/ml (median, 87 ng/ml). Compared with population controls, cases had significantly higher caloric intake, had significantly smaller hips and larger waist:hip ratios, and were somewhat less likely to be married, have had attended college, or be smokers or drinkers, although not significantly so (data not shown).

Table 1 shows the allele frequencies of each of the four markers by case-control status. We did not observe the T allele of the *A49T* marker among cases or controls, and for the *R227Q* marker, only three subjects (two cases and one control) possessed the Q allele. For the (TA)<sub>n</sub> repeat marker, the (TA)<sub>o</sub> allele was less frequent among cases than controls (6.5% versus 9.1%), and the only subject carrying the (TA)<sub>18</sub> allele was a control.

Table 2 shows the distribution of genotypes for the four markers and age-adjusted ORs for prostate cancer in relation to these genotypes. Inclusion of any/all of the potential risk factors in regression models did not appreciably affect risk. Because all study subjects had the *AA* genotype for the *A49T* marker, no ORs could be estimated for this polymorphism. For the *V89L* marker, the heterozygous *VL* genotype was the most common (45%), followed by the homozygous *LL* genotype (35%) and the homozygous *VV* genotype (21%). Compared with men with the *VV* genotype, those with the *LL* genotype had a nonsignificant 12% reduced risk (OR, 0.88; 95% CI, 0.53–1.47). There

Table 1 Allele frequency of polymorphic markers in the *SRD5A2* gene

Allele frequency	Cases (n = 191)	Controls (n = 304)
<i>A49T</i> <sup>a</sup>		
A	100.0	100.0
T	0.0	0.0
<i>V89L</i> <sup>b</sup>		
L	55.4	57.1
V	44.6	42.9
<i>R227Q</i> <sup>c</sup>		
Q	0.6	0.2
R	99.4	99.8
(TA) <sub>n</sub> <sup>d</sup>		
(TA) <sub>o</sub>	93.5	90.8
(TA) <sub>9</sub>	6.5	9.1
(TA) <sub>18</sub>	0.0	0.2

<sup>a</sup> 170 cases and 256 controls.

<sup>b</sup> 186 cases and 303 controls.

<sup>c</sup> 176 cases and 268 controls.

<sup>d</sup> 191 cases and 304 controls.

was no association of the *VL V89L* genotype with risk. For the *R227Q* marker, compared with men with the *RR* genotype, those with the *RQ* genotype had a nonsignificantly elevated risk (OR, 2.85; 95% CI, 0.25–32.1), although this result is based on two cases and a single control subject.

The (TA)<sub>o</sub>/(TA)<sub>o</sub> genotype was the most common genotype of the (TA)<sub>n</sub> repeat polymorphism, and the heterozygous (TA)<sub>o</sub>/(TA)<sub>9</sub> genotype was found to be less common among cases than controls (12% versus 17%). Because the (TA)<sub>18</sub> allele was observed only once in this population [in a (TA)<sub>o</sub>/(TA)<sub>18</sub> control], it was combined with the (TA)<sub>9</sub> allele for logistic regression analysis. Men with the (TA)<sub>o</sub>/(TA)<sub>9</sub> genotype had a modestly reduced risk compared with men homozygous for the (TA)<sub>o</sub> allele (OR, 0.67; 95% CI, 0.39–1.12).

Age-adjusted mean serum levels of the sex hormones and SHBG by genotypes of *V89L* and (TA)<sub>n</sub> repeats are shown in Table 3. Serum levels of T and 3 $\alpha$ -diol G (but not DHT, estradiol, or SHBG) were significantly different across genotypes of the *V89L* polymorphism (*P* = 0.007 and *P* = 0.002, respectively). Men with the *VL* and *LL* genotypes had similar levels of both T and 3 $\alpha$ -diol G. Compared with men with the *VV* genotype, men with the *VL* and *LL* genotypes had higher (17%) levels of T and lower (24%) levels of 3 $\alpha$ -diol G. The (TA)<sub>o</sub>/(TA)<sub>9</sub> genotype of the (TA)<sub>n</sub> repeat length polymorphism was significantly associated with a 14% higher serum DHT level compared with men with the (TA)<sub>o</sub>/(TA)<sub>o</sub> genotype (*P* = 0.03). No association of the (TA)<sub>n</sub> polymorphism with serum T, 3 $\alpha$ -diol G, estradiol, or SHBG levels was observed.

## Discussion

We found no statistically significant associations with prostate cancer risk of the *A49T*, *V89L*, *R227Q*, and (TA)<sub>n</sub> polymorphisms in the *SRD5A2* gene, although a modest effect of the (TA)<sub>n</sub> and *V89L* markers cannot be ruled out. The *A49T* T allele and the *R227Q* Q allele, which, along with the long (TA)<sub>n</sub> allele, have been linked to increased 5 $\alpha$ -reductase enzyme activity in laboratory studies, were observed at very low frequencies, if at all. However, we found that men with the *VV V89L* genotype had significantly higher levels of 3 $\alpha$ -diol G than men with other *V89L* genotypes, whereas men with the (TA)<sub>o</sub>/(TA)<sub>9</sub> (TA)<sub>n</sub> genotype had higher DHT levels compared with men with the (TA)<sub>o</sub>/(TA)<sub>o</sub> genotype, suggesting that these markers may potentially influence prostate cancer risk despite the nonsignifi-

Table 2 ORs<sup>a</sup> for prostate cancer in relation to polymorphic markers in the *SRD5A2* gene

Genotype	Cases		Controls		OR <sup>a</sup>	95% CI
	N	%	N	%		
<i>A49T</i>						
AA	170	100.0	256	100.0		
<i>V89L</i>						
VV	40	21.5	62	20.5	1.00	
VL	86	46.2	136	44.9	0.98	0.60–1.58
LL	60	32.3	105	34.7	0.88	0.53–1.47
<i>R227Q</i>						
RR	174	98.9	267	98.4	1.00	
RQ	2	1.1	1	1.6	2.85	0.25–32.1
<i>(TA)<sub>n</sub></i>						
(TA) <sub>0</sub> /(TA) <sub>0</sub>	167	87.4	250	82.2	1.00	
(TA) <sub>0</sub> /(TA) <sub>0</sub>	23	12.0	51	16.8	0.67 <sup>b</sup>	0.39–1.12
(TA) <sub>0</sub> /(TA) <sub>18</sub>	0	0.0	1	0.3		
(TA) <sub>0</sub> /(TA) <sub>0</sub>	1	0.5	2	0.7	0.74	0.07–8.31

<sup>a</sup> Adjusted for age.<sup>b</sup> OR was calculated including the single control possessing the (TA)<sub>0</sub>/(TA)<sub>18</sub> genotype.Table 3 Means<sup>a</sup> and 95% CIs of serum hormone levels among population controls by genotypes of the *V89L* and *(TA)<sub>n</sub>* polymorphisms of the *SRD5A2* gene

<i>SRD5A2</i> marker	N	T (ng/dl)	DHT (ng/dl)	3 $\alpha$ -diol G (ng/dl)	Estradiol (ng/dl)	SHBG (nmol/liter)
<i>V89L</i>						
VV	62	570 (499–641) <sup>b</sup>	62.1 (53.0–71.2) <sup>b</sup>	761 (641–882)	4.84 (4.39–5.28)	33.7 (27.1–40.3)
VL	136	672 (613–732)	71.2 (63.5–78.8)	573 (471–674)	5.06 (4.68–5.43)	37.3 (31.8–42.9)
LL	105	659 (596–723)	66.9 (58.7–75.1)	584 (477–693)	5.03 (4.63–5.43)	33.7 (27.8–39.6)
p <sup>c</sup>		0.007	0.10	0.002	0.55	0.29
<i>(TA)<sub>n</sub></i>						
(TA) <sub>0</sub> /(TA) <sub>0</sub>	250	635 (580–691)	65.7 (58.6–72.7)	621 (526–716)	4.97 (4.63–5.32)	34.6 (29.5–39.7)
(TA) <sub>0</sub> /(TA) <sub>0</sub>	51	675 (601–749)	75.1 (65.8–84.5)	603 (477–729)	5.06 (4.60–5.52)	37.6 (30.9–44.4)
p <sup>c</sup>		0.23	0.03	0.75	0.67	0.32

<sup>a</sup> Age-adjusted mean.<sup>b</sup> 95% CIs are shown in parentheses.<sup>c</sup> P from F-test for overall differences in analyte levels by genotype.

cant risk estimates in our study. Consistent with earlier reports, our data show that the *LL* genotype of the *V89L* marker is common in Chinese men (35%), that (TA)<sub>0</sub>/(TA)<sub>0</sub> is the most common genotype of the *(TA)<sub>n</sub>* marker, and that the *R227Q* marker's rare *RQ* genotype, which is associated with reduced steroid 5 $\alpha$ -reductase activity and has been detected only in Asians, is present in this Chinese population (16, 17, 19).

Of the more than 20 mutations reported for the *SRD5A2* gene, *V89L* is the most common (16). Consistent with Febbo *et al.* (19) and Nam *et al.* (32), we found a slight nonsignificant reduction in risk of prostate cancer associated with the *LL* genotype (12%), although two studies conducted among Western and Japanese men did not find such an association (20, 21). Reasons for this inconsistency are unclear, but they may be due in part to the use of benign prostatic hyperplasia patients as controls in one null study (21). Recent studies show that distributions of *V89L* genotypes parallel the patterns of prostate cancer incidence in high- and low-risk populations, with *LL* genotype prevalences of 22–25% among Asians and of only about 4% among Caucasian and African Americans and *VV* genotype prevalences of 27–29% among Asians and of about 58% among African and Caucasian Americans (16, 21). Our data are consistent with these results: among controls, 35% had the *LL* genotype, and only 21% had the *VV* genotype. The prevalence among controls of the *L* allele was somewhat higher in this Chinese population than has been reported previously in a group of Chinese and Japanese men (57% versus 46%; Ref.

15). Although the risk reductions associated with the *LL* genotype in our study and in that of Febbo *et al.* (19) were nonsignificant, given the large racial differences in *V89L* genotype distributions and the marker's potential influence on 5 $\alpha$ -reductase enzyme activity, a small effect of the *V89L* polymorphism on prostate cancer risk cannot be ruled out. Larger studies are needed to elucidate whether the *V89L* polymorphism is associated with a modest elevation in prostate cancer risk.

Recent studies show that, compared with the *VV* genotype, the *LL V89L* genotype confers a 42% reduction in 5 $\alpha$ -reductase enzymatic activity *in vitro* (11). In addition, compared with the *VV* genotype, the *LL* genotype is associated with lower serum 3 $\alpha$ -diol G levels among Asian (15) and British (33) men, although the difference was not significant among British men, possibly due to the low observed prevalence of the *LL* genotype (8%; Ref. 33). Similarly, in our study of Chinese men, the *LL* genotype is associated with significantly lower serum 3 $\alpha$ -diol G levels compared with the *VV* genotype, suggesting lower 5 $\alpha$ -reductase enzymatic activity among men with the *LL* genotype. The significantly higher serum T levels among men with the *LL* genotype (compared with men with the *VV* genotype) also suggests lower 5 $\alpha$ -reductase activity, because within the prostate, lower 5 $\alpha$ -reductase activity will result in less conversion of T to DHT, and the accumulating T within the prostate may leak back into circulation to raise serum levels. However, the lack of a complementary low serum DHT level among men with the *LL* genotype seems inconsistent with this possibility,



which must thus be examined further. In addition, the dominant influence of the *L* allele on 5 $\alpha$ -reductase enzyme activity suggested by the similar 3 $\alpha$ -diol G levels among men with the *VL* and *LL* genotypes in our study, but not a previous one (33), supports *in vitro* data showing that the *L* allele is partial dominant and that there is almost no difference in enzyme activity between the *VL* and *LL* genotypes (11).

Despite the low frequency of the *A49T* *T* allele among healthy subjects in most populations, this allele is implicated in prostate cancer for several reasons: (a) an earlier study found almost 10% of the prostate cancer cases had the *T* allele (12); (b) the *T* allele was linked to progression and aggressiveness of prostate tumors in a pathologic survey (18); and (c) of all known mutations in the *SRD5A2* gene, this substitution increased *in vitro* 5 $\alpha$ -reductase enzymatic activity the most (almost 5-fold; Ref. 11). Earlier studies reported that only 0.5% of African Americans and 1.8% of Latino men harbored this allele (16, 18). In our study, we found no subjects with the *T* allele. Because of the rarity of the *A49T* *T* allele and its substantial impact on 5 $\alpha$ -reductase activity, larger studies are needed to further clarify its role in the development of prostate cancer.

The *Q* allele of the *R227Q* polymorphism, like the *T* allele of the *A49T* marker, also substantially reduces 5 $\alpha$ -reductase activity *in vitro*, and subjects with the *RQ* and *QQ* genotypes have reduced enzymatic activity compared with those with the *RR* genotype (11). *RR* is the most common genotype in the healthy population; subjects with the *QQ* genotype are male pseudohermaphrodites (34), and to date, the *RQ* genotype has been reported in Asians only (11). In our study, we found no subjects with the *QQ* genotype. We are able to confirm that the *Q* allele was present in three heterozygotes (one control and two cases) but were unable to show a significant association with prostate cancer because of the rarity of the *Q* allele.

Because the (*TA*)<sub>*n*</sub> marker is located in the 3'-UTR of the *SRD5A2* gene, its functional consequences are thought to be due to the instability of mRNA transcripts with UA-rich 3'-UTRs [transcribed from TA-rich regions of DNA (16, 35)], which may in turn affect 5 $\alpha$ -reductase activity levels. The relevance of the (*TA*)<sub>*n*</sub> marker in prostate cancer is further supported by the finding that 56% of 30 prostate tumors possessed somatic mutations at this locus (36). Three major (*TA*)<sub>*n*</sub> alleles have been reported, namely, (*TA*)<sub>6</sub>, (*TA*)<sub>9</sub>, and (*TA*)<sub>18</sub>, with (*TA*)<sub>6</sub> being the most common in most populations. In our study, 84% of the controls had the (*TA*)<sub>6</sub>/*(TA)*<sub>6</sub> genotype. Consistent with Kantoff *et al.* (22), we found that men carrying copies of the longer (*TA*)<sub>*n*</sub> allele [all but one had the (*TA*)<sub>6</sub>/*(TA)*<sub>9</sub> genotype] had a nonsignificantly lower risk of prostate cancer. We found that serum levels of DHT, but not those of other sex hormones, were higher among men with the (*TA*)<sub>6</sub>/*(TA)*<sub>9</sub> genotype, although the biological significance of this is unclear. Finally, because only one subject in our study possessed the rare (*TA*)<sub>18</sub> allele, we were unable to evaluate the effect of this long allele.

Rare, highly penetrant genetic factors that confer a high relative risk on the few individuals who carry them (for example, *HPC1* on chromosome 1) may explain about 10% of the prostate cancer cases in the United States and are unlikely to explain the large racial/ethnic differences in prostate cancer risk. In contrast, common polymorphisms confer variable risk upon all individuals, which may, in turn, result in a much larger proportion of prostate cancer cases attributed to possession of certain genotypes. Although we found no statistically significant association with polymorphisms of *SRD5A2*, a small effect cannot be ruled out. Indeed, common polymorphisms of several susceptibility genes involved in androgen metabolism or signal

transduction within the prostate have recently been linked to prostate cancer, including the *AR*, *AIB1*, *CYP17*, and *HSD3B2* genes (12, 24, 37–39).<sup>4</sup> It is possible that a set of such markers might together alter androgenic action within the prostate gland, thus heightening prostate cancer risk in a subset of individuals. Future studies should examine the combined effects of these polymorphisms to provide insights into the origins of prostate cancer.

Biases arising from survival and selection should be minimal in our study because well over 90% of the eligible cases participated in the study and most cases were interviewed within 30 days of diagnosis. Furthermore, 70–80% of the study subjects gave blood for the study; thus it is unlikely that response status among cases and controls was related to the observed allele frequencies.

This is the first population-based effort in a low-risk population to examine the role of *SRD5A2* in prostate cancer. Although we found no statistically significant associations of these four polymorphic markers with prostate cancer risk, a modest effect cannot be ruled out. Furthermore, the *V89L* and (*TA*)<sub>*n*</sub> markers were associated with serum androgen levels, supporting a role of these markers in prostate cancer etiology. Larger studies, including those on African Americans, are needed to further clarify the role of *SRD5A2* and type II 5 $\alpha$ -reductase in prostate cancer and determine whether they can explain the large racial/ethnic difference in prostate cancer risk.

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